CONCISE COMMUNICATION

Lack of Association of Hepatitis C Virus Load and Genotype with Risk of End-Stage Liver Disease in Patients with Human Immunodeficiency Virus Coinfection

James J. Goedert,[1] Angelos Hatzakis,[4] Kenneth E. Sherman,[2] and M. Elaine Eyster,[3] for the Multicenter Hemophilia Cohort Study

[1]Viral Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland; [2]Division of Digestive Diseases, University of Cincinnati Medical Center, Cincinnati, Ohio; [3]Department of Medicine, Division of Hematology/Oncology, Pennsylvania State University College of Medicine, Hershey; [4]National Retrovirus Reference Center, Department of Hygiene and Epidemiology, Athens University, Athens, Greece

In hepatitis C virus (HCV) infection, virus load and the risk for HCV-related end-stage liver disease (ESLD) are increased among persons with human immunodeficiency virus (HIV) coinfection. To clarify these relationships, 42 hemophilic patients who developed ESLD and random samples from 164 hemophilic patients with HCV infection alone and 146 with HCV-HIV coinfection were tested for HCV load and genotype. HCV genotype was unrelated to HIV and age. In contrast, HCV load was higher with older age ($P_{trend} = .0001$) and with HIV coinfection (6.2 vs. 5.9 $\log_{10}$ genome equivalents/mL, $P = .0001$). During 16 years of follow-up of dually infected patients, ESLD risk was unrelated to HCV load overall ($P_{trend} = .64$) or separately to HCV genotype 1 and genotypes 2 or 3 ($P_{trend} \geq .70$). Irrespective of virus load, incidence of ESLD was marginally increased 2-fold (95% confidence interval, 0.8–5.6) with HCV genotype 1. Understanding the discordance between HCV load and ESLD, despite HIV's link to each of these, may help clarify the pathogenesis of HCV-related disease.

By the mid-1980s, about half of persons with hemophilia were infected with both hepatitis C virus (HCV) and human immunodeficiency virus (HIV) and an additional one-quarter to one-third were infected with HCV but not HIV [1]. About 80% of such patients develop persistent HCV infection, manifested as detectable viremia with or without chronic hepatic aminotransferase elevations [2]. End-stage liver disease (ESLD) is the major outcome of chronic HCV infection. Risk of ESLD, particularly for older hemophilic patients, appears to be increased with HIV coinfection [1, 3, 4] and may relate to an aberrant immune response to HCV infection [5]. Here, we investigated whether HCV genotype or virus load was predictive of ESLD in a prospectively followed cohort of HCV-HIV coinfected patients with hemophilia.

Received 25 May 2001; revised 16 July 2001; electronically published 13 September 2001.

Informed consent was obtained from the patients or their parents or guardians, and human experimentation guidelines of the US Department of Health and Human Services and those of the authors' institutions were followed in the conduct of clinical research.

Financial support: National Cancer Institute (CP-33002) in association with Research Triangle Institute.

Reprints or correspondence: Dr. James Goedert, 6120 Executive Blvd., Ste. 8012, MSC 7248, Rockville, MD 20852 (GoedertJ@mail.nih.gov).

The Journal of Infectious Diseases 2001; 184:1202–5

0022-1899/2001/18409-0017$02.00

Methods

Participants and specimens. The distributions of HCV load and genotypes were examined in stratified random samples of HCV-HIV coinfected and HCV-positive, HIV-negative participants of the Multicenter Hemophilia Cohort Study (MHCS): 20 participants each were from 8 birth cohorts (1 cohort consisting of those born before 1950, six 5-year cohorts of those born between 1950 and 1979, and 1 cohort of those born after 1979) [6]. Only MHCS centers in the United States were included. ESLD was defined as persistent ascites, bleeding esophageal varices, hepatic encephalopathy, or death, excluding nonhepatic causes [1]. The random samples did not differ from the MHCS US population in severe hemophilia A prevalence ($P = .80$), cumulative 16-year survival rate ($P = .44$), or ESLD incidence rate ($P = .51$; data not shown). Of the participants in the random samples, 310 (97%) had sufficient reposited specimens for determination of HCV load and genotype. In addition to the 12 ESLD case patients in the coinfected random sample, the 30 other coinfected US ESLD case patients with a prediagnostic specimen were included for case-cohort analysis of ESLD risk [7]. Differences over time in HCV load by HIV and ESLD status were evaluated by testing a second specimen of the same type (serum or plasma) that was obtained ~5 years earlier.

Virus load and genotype testing. HCV load was determined with branched DNA (bDNA) technology (Quantiplex HCV RNA 2.0 assay, Chiron) with a lower limit of sensitivity of 200,000 (5.3 $\log_{10}$) genome equivalents (GEq)/mL. HCV load was transformed to $\log_{10}$ for all analyses, and, for continuous analyses, it was input as 100,000 (5.0 $\log_{10}$) GEq/mL for bDNA-negative specimens. For

genotyping, HCV RNA was reverse transcribed and amplified by the polymerase chain reaction (PCR), using nested biotinylated primers to the highly conserved 5′ noncoding region developed in our laboratory (A.H.) and/or using a commercially available HCV RNA assay (Amplicor HCV, Roche Diagnostic Systems). Genotyping of the PCR products was done on the labeled amplicons, using a line-probe assay (LiPA, version 1.0; Immunogenetics) [8]. Specimens with no PCR product for the LiPA were considered to be PCR negative. Specimens with atypical band patterns were referred to as genotype unspecified. The rest were grouped into major genotypes/subtypes (HCV-1a, -1b, -2, -3, or -4).

Statistical analysis. The coinfected cohort was followed, on average, for 16 years from individual input HIV seroconversion dates [6] to the date of the onset of ESLD, death, or last follow-up (typically in late 1998). Use of alternate starting dates, such as individual birth dates or the median HIV seroconversion date for the MHCS as a whole, had no substantive effect on the results (data not presented). Proportional hazards modeling (Epicure, HiroSoft International) for a case-cohort study [7] was used to determine the relative hazard and 95% confidence interval of ESLD by HCV genotype and virus load, adjusted for age. The relationship of HCV load to HIV status, genotype, hemophilia type and severity, and age was examined by analysis of variance. A Student's *t* test was used to compare change in HCV load by HIV or ESLD status. The distribution of genotypes by birth cohort and HIV status was tested by χ^2 and Fisher's exact tests.

Results

Of the 310 randomly selected US hemophilic participants, 173 (56%) had HCV genotype 1, including 92 (30%) with subtype 1a and 81 (26%) with subtype 1b. Thirty-two participants had genotype 2, 30 had genotype 3, and 3 had genotype 4. Seven participants (1% of HIV-negative and 3% of HIV-positive participants) had multiple genotypes detected, 17 (5%) had genotypes that could not be specified, and 48 (15%) were HIV negative, as determined by PCR. Genotypes did not differ by birth cohort or HIV status (table 1), but significantly more HIV-negative than HIV-positive participants had PCR-negative results (23% vs. 8%, $P = .0001$).

HCV load was higher among HIV-positive, compared with HIV-negative, participants (6.2 vs. 5.9 $\log_{10}$ GEq/mL, $P = .0001$), and, over a mean of 4.9 years, it increased even more (0.42 vs. 0.16 $\log_{10}$ GEq/mL, $P = .0002$). HCV load also was higher with older age, for both the HIV-negative participants (0.017 $\log_{10}$ GEq/mL per year, $P = .0001$) and the HIV-positive participants (0.018 $\log_{10}$ GEq/mL per year, $P = .0001$) (figure 1).

Irrespective of HIV status, mean HCV load was 6.3 $\log_{10}$ GEq/mL with HCV types 1a, 1b, and 2, slightly lower (6.1 $\log_{10}$ GEq/mL) with genotype 3, and nonsignificantly higher (6.6 $\log_{10}$ GEq/mL) among the 7 participants with multiple genotypes. Likewise, mean virus loads were 6.1 $\log_{10}$ GEq/mL in participants with mild, moderate, or severe hemophilia A or with factor VIII inhibitors. HCV load was lower (5.8 $\log_{10}$ GEq/mL) among participants with hemophilia B, but this difference was not significant with adjustment for HIV status and age (data not shown).

Risk of ESLD with HCV-HIV coinfection appeared to be 2-fold higher with genotype 1 than with genotype 2 or 3 ($P = .09$; table 1). ESLD risk was unrelated to HCV load overall

Table 1. Hepatitis C virus (HCV) genotypes and virus load, by human immunodeficiency virus (HIV) status in relation to prevalence and risk of end-stage liver disease (ESLD).

Variable	No. of participants[a]		No. of HIV-positive case patients with ESLD	Adjusted relative hazard (95% CI)[b]	P[c]
	HIV negative ($n = 164$)	HIV positive ($n = 146$)			
HCV subtype or genotype					
1a	38	54	21	1.0 (referent)	
1b	46	35	11	0.9 (0.4–2.3)	
2	13	19	3	0.4 (0.1–1.7)	
3	17	13	3	0.5 (0.1–2.1)	.63
4	1	2	0	Not evaluable	
Multiple	2	5	0	Not evaluable	
Negative or unspecified	47	18	4	0.7 (0.2–2.4)	
HCV genotype					
1	84	89	32	2.0 (0.8–5.6)	
2 or 3	30	32	6	1.0 (referent)	.09
HCV load, $\log_{10}$ GEq/mL					
<5.3[d]	63	27	7	1.0 (referent)	
5.3–5.99	26	23	10	0.9 (0.3–3.0)	.64 (trend)
6.0–6.99	56	68	13	0.6 (0.2–1.7)	
7.0	19	28	12	0.9 (0.3–2.8)	

NOTE. CI, confidence interval; GEq, genome equivalents.

[a] Stratified random sample of US hemophilic population.

[b] Comparison of ESLD case patients with HIV-positive participants without ESLD, adjusted for age, using 3 strata: 0–16, 17–32, and 33–86 years.

[c] Log-rank tests for genotype; 1*df* trend test for HCV load.

[d] Limit of sensitivity of the assay.

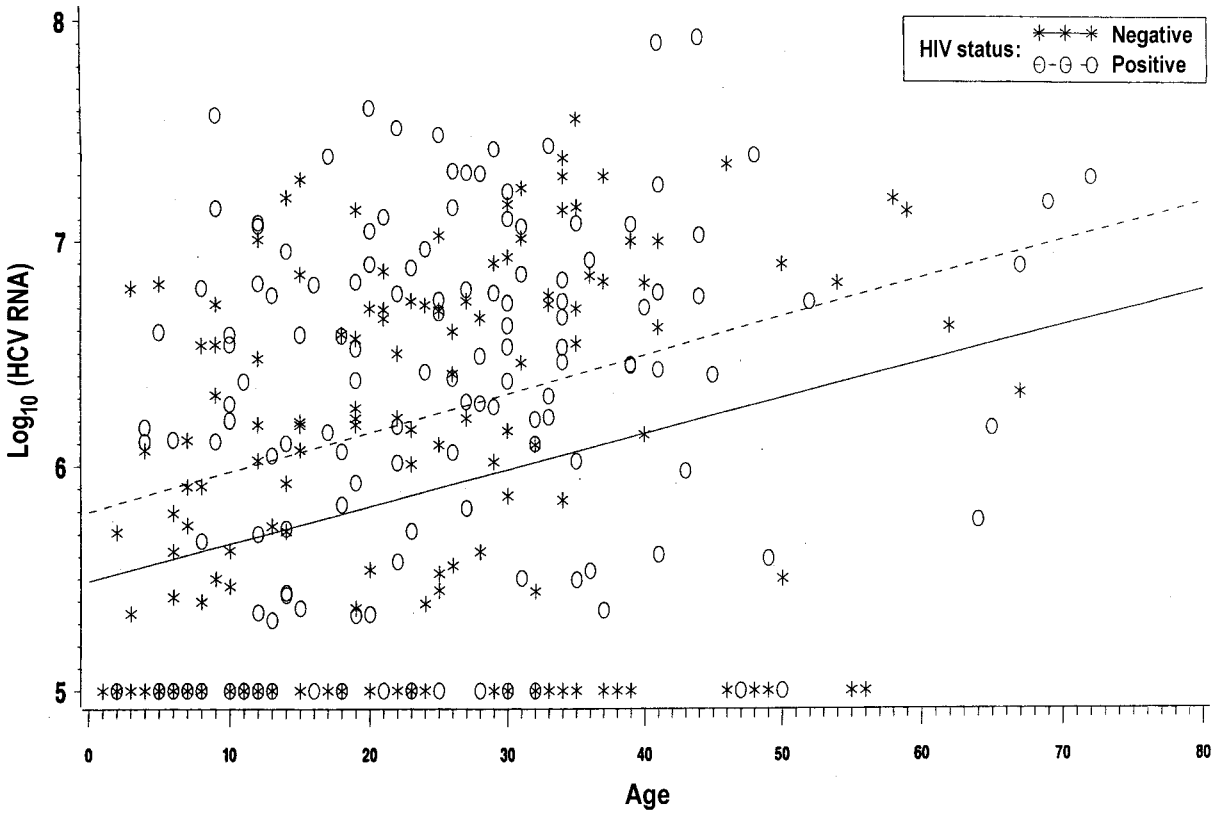

Figure 1. Hepatitis C virus (HCV) load among participants of the Multicenter Hemophilia Cohort Study, by age (at time of sampling) and human immunodeficiency virus (HIV) infection status. Virus load was higher in HIV-positive than in HIV-negative subjects and increased with age in both groups. HCV RNA was measured by branched DNA (bDNA) technology; specimens with no detectable viremia, by polymerase chain reaction, were excluded. For reference, 6.0 $\log_{10}$ is 10^6 viral genome equivalents (GEq)/mL. The lower limit of detection is 5.3 $\log_{10}$ (200,000 GEq/mL); specimens with levels not detected by bDNA were set to 5.0 $\log_{10}$ (100,000 GEq/mL).

(P_{trend} = .64; table 1) and in subgroups with HCV genotype 1 or genotypes 2 or 3 (P_{trend} = .70 and .81, respectively). Over a mean interval of 4.9 years between paired samples, HCV load increased 0.48 $\log_{10}$ GEq/mL in coinfected participants who developed ESLD, compared with 0.41 $\log_{10}$ GEq/mL in coinfected participants who did not develop ESLD (P = .61).

Discussion

ESLD in an HCV-HIV coinfected patient with hemophilia is a major clinical event that often leads rapidly to death [1, 4]. HCV-related liver fibrosis and, presumably, ESLD are associated with duration of infection, older age, male sex, high alcohol consumption, HIV infection, and low $CD4^+$ lymphocyte count [2, 9]. We found recently that ESLD risk was increased with an atypical HCV antibody pattern (high anti-c100 and low anti-c22) [5]. We studied 310 patients who are highly representative of the US hemophilia population, to clarify the interaction of HIV with HCV genotypes, virus load, and ESLD risk.

Overall, with or without HIV, we found no relationship between HCV load and genotype, as noted usually but not always [3, 10, 11]. The associations noted between HCV load and HIV, including a significantly higher prevalence of HCV viremia and a nonsignificantly higher prevalence of multiple genotypes, deserve further study. HIV-related immune dysfunction presumably impairs clearance of HCV, but the components involved are unknown. Detection of multiple genotypes, as well as switches in genotypes over time [3, 8], in this repeatedly exposed population were not limited to those with HIV and may be a function of HCV load, which was 0.3 $\log_{10}$ GEq/mL higher than in those with a single HCV genotype. The distribution of genotypes that we found was similar to that found in a previous study [12]. In addition, our representative sampling enabled us to show that HCV genotypes were independent of birth cohort, suggesting that there were no large genotype shifts in the plasma donor population before the mid-1980s.

As in the study by Thomas et al. [13], HCV load in our study was higher with older age. In addition, we and others found that HCV load was much higher and increased more steeply over time with HIV coinfection [3, 13, 14]. Despite this association, age-adjusted ESLD risk was unrelated to HCV load in the coinfected participants overall and in the coinfected subgroups with HCV genotype 1 or with genotypes 2 or 3. As in

other studies [3, 10, 11], our power to investigate subgroups was limited except with genotype 1. Irrespective of virus load, the risk of ESLD was nonsignificantly increased 2-fold for coinfected participants with HCV genotype 1. In contrast, in a recent multivariate analysis of 125 HCV-HIV coinfected and 185 HCV singly infected hemophilic patients, genotype 1 was associated with a 7.8-fold (95% confidence interval, 2.0–30.8) increased risk of liver death [4]. Additional studies will be needed to reconcile these findings. We found no difference in ESLD risk between HCV subtypes 1a and 1b.

In summary, HIV infection is associated with increased HCV load, with an increased rate of liver fibrosis [9], and with ESLD [1, 3, 4]. Despite these links, we found that HCV load was not associated with ESLD, even in subgroups defined by HCV genotype. This paradox is quite unlike the straightforward association between HIV load and AIDS, perhaps reflecting the relationship between liver fibrosis and intrahepatic T lymphocyte cytotoxicity, which appears to be unrelated to HCV load [15]. These and other such studies are likely to lead to improved understanding of the pathogenesis of HCV-related diseases and their treatment and prevention.

Acknowledgements

We thank Brad Goebel, Mike Walters, and Andrea Stossel (Scientific Applications International Corporation), for performing the assays; Susan Wilson (Research Triangle Institute) and Frances Yellin (Computer Sciences Corporation), for computer programming; and Virginia Lamprecht and Barbara Kroner (Research Triangle Institute), for study management. We are especially grateful to the study participants, hemophilia center staff members, and collaborators of the Multicenter Hemophilia Cohort Study for their tireless contributions.

References

1. Eyster ME, Diamondstone LS, Lien JM, Ehmann WC, Quan S, Goedert JJ. Natural history of hepatitis C virus infection in multitransfused hemophiliacs: effect of coinfection with human immunodeficiency virus. J Acquir Immune Defic Syndr **1993**;6:602–10.
2. Seeff LB. Natural history of hepatitis C. Am J Med **1999**;107:10S–5S.
3. Eyster ME. Effect of HIV infection on hepatitis C. Viral Hepatitis Rev **1998**;4:189–206.
4. Yee TT, Griffioen A, Sabin CA, Dusheiko G, Lee CA. The natural history of HCV in a cohort of haemophilic patients infected between 1961 and 1985. Gut **2000**;47:845–51.
5. Goedert JJ, Hatzakis A, Maloney EM, Eyster ME. Increased liver decompensation risk with atypical hepatitis C virus antibody levels. J Infect Dis **2000**;182:590–4.
6. Kroner BL, Rosenberg PS, Aledort LM, Alvord WG, Goedert JJ. HIV-1 infection incidence among persons with hemophilia in the United States and Western Europe, 1978–1990. J Acquir Immune Defic Syndr **1994**;7:279–86.
7. Wacholder S, Gail M, Pee D. Selecting an efficient design for assessing exposure-disease relationships in an assembled cohort. Biometrics **1991**;47:63–76.
8. Eyster ME, Sherman KE, Goedert JJ, Katsoulidou A, Hatzakis A. Prevalence and changes in hepatitis C virus genotypes among multitransfused persons with hemophilia. The Multicenter Hemophilia Cohort Study. J Infect Dis **1999**;179:1062–9.
9. Benhamou Y, Bouchet M, Di Martino V, et al. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. Hepatology **1999**;30:1054–8.
10. Ahmed MM, Mutimer DJ, Martin B, Elias E, Wilde JT. Hepatitis C viral load, genotype and histological severity in patients with bleeding disorders. Haemophilia **1999**;5:49–55.
11. Perez-Gracia T, Galan F, Fernandez-Gutierrez C, Giron JA, Rodriguez-Iglesias M. Relationship of hepatitis C viremia to HIV state and to infection by specific hepatitis C genotypes. Liver **1999**;19:288–93.
12. Lau JYN, Mizokami M, Kolberg JA, et al. Application of six hepatitis C virus genotyping systems to sera from chronic hepatitis C patients in the United States. J Infect Dis **1995**;171:281–9.
13. Thomas DL, Astemborski J, Vlahov D, et al. Determinants of the quantity of hepatitis C virus RNA. J Infect Dis **2000**;181:844–51.
14. Eyster ME, Fried MW, Di Bisceglie AM, Goedert JJ. Increasing hepatitis C virus RNA levels in hemophiliacs: relationship to human immunodeficiency virus infection and liver disease. The Multicenter Hemophilia Cohort Study. Blood **1994**;84:1020–3.
15. Nelson DR, Marousis CG, Davis GL, et al. The role of hepatitis C virus–specific cytotoxic T lymphocytes in chronic hepatitis C. J Immunol **1997**;158:1473–81.